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Award Number:
W81XWH-09-1-0310

TITLE:
Achilles Heel of ETS Fusions

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REPORT DATE:

July 2010

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-07-2010	Annual	1 JUL 2009 - 30 JUN 2010

4. TITLE AND SUBTITLE
Achilles Heel of ETS fusions

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0310

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Stanford, California 94010

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
In prostate cancer TRMPRSS2-ETS fusions are common, but not readily druggable. Our study aims to discover novel therapeutic targets displaying synthetic lethal (or Achilles' heel) interaction with ETS fusions, using a highly multiplexed RNA interference-based approach. Towards this goal, key accomplishments during the first year include the creation and validation of ETS knockdown and inducible prostate cell lines; the piloting and optimization of pooled shRNA barcode screens; and the completion of the first whole-genome shRNA screens. Future efforts will focus on the completion of screens, analysis of data, and validation of positive hits as genes that are selectively targetable in the context of ETS fusions.

15. SUBJECT TERMS
TMPRSS2-ERG fusion, RNA interference, drug target identification, synthetic lethality

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	7	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Page

Introduction

Prostate cancer is a leading cause of cancer death, highlighting the need for new molecular targets and therapies. An important recent discovery was that more than half of prostate cancers express a gene fusion typically between TMPRSS2 and an oncogenic ETS family transcription factor (i.e. ERG, ETV1, ETV4 or ETV5). However, transcription factors are notoriously difficult to target. Nonetheless, prostate cancer cells expressing ETS fusion genes might exist in specific genetic contexts that are exploitable via "synthetic lethal" interactions.

The goal of our study is to discover novel therapeutic targets displaying synthetic lethal (or Achilles' heel) interaction with ETS fusions, using a highly multiplexed RNA interference-based approach. Specifically, we have proposed three tasks: (1) To carry out pooled shRNA viability screens comparing prostate cancer cell lines with and without ETS fusions (months 1-12); (2) To analyze resultant data and identify genes and pathways exhibiting synthetic lethality with ETS fusions (months 13-14); and (3) To validate synthetic lethality of identified genes and pathways for follow-up mechanistic and therapeutic studies (months 15-24).

Body

Task 1A:
We originally proposed to complete the construction of an isogenic VCaP prostate cancer line with stable knockdown of TMPRSS2-ERG. In working towards this goal, we found that VCaP cells grow extremely slowly, with a doubling time of greater than one week. Thus, using this cell line for an "outgrowth" screen that requires many population doublings to identify depleted shRNAs would not be practical. We have therefore constructed an alternative cell line, an isogenic LNCaP cell line with stable knockdown of ETV1 (the ETS oncoprotein expressed in this line). Screening 6 different shRNAs targeting ETV1, we have validated knockdown in 2 (Fig. 1).

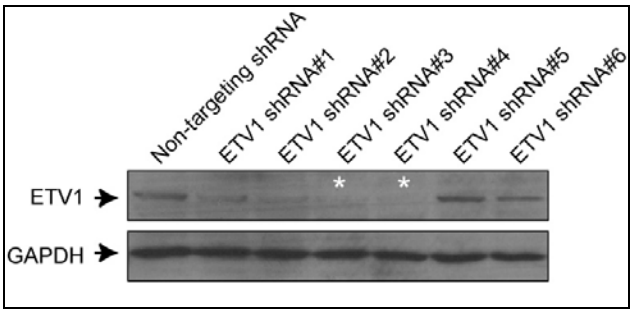

Fig 1. Isogenic LNCaP cell lines with stable knockdown of ETV1. Western blot validates knockdown of ETV1 by shRNAs targeting ETV1 (>90% knockdown denoted by asterisks), compared to a non-targeting control shRNA. GAPDH serves as a loading control.

In addition to constructing the above cell line, we have created another useful cell line for our screen. We had previously created an RWPE1 prostate cell line with conditional expression of TMPRSS2-ERG (shown as preliminary data in the original submission). However, we noted that the original prostate tumor from which that cDNA was cloned actually expressed two different transcript variants. By sequencing analysis, we have determined that the larger variant (variant 2) contains an additional exon, which we have designated exon '7B' with respect to the RefSeq transcript (Fig. 2A). Since it was uncertain which transcript variant was more functionally relevant, we have now engineered a new RWPE1 line with inducible expression of variant 2. Both variants enhance invasion of RWPE1 cells (Fig. 2B), and this new line now serves as an additional resource for our shRNA screen.

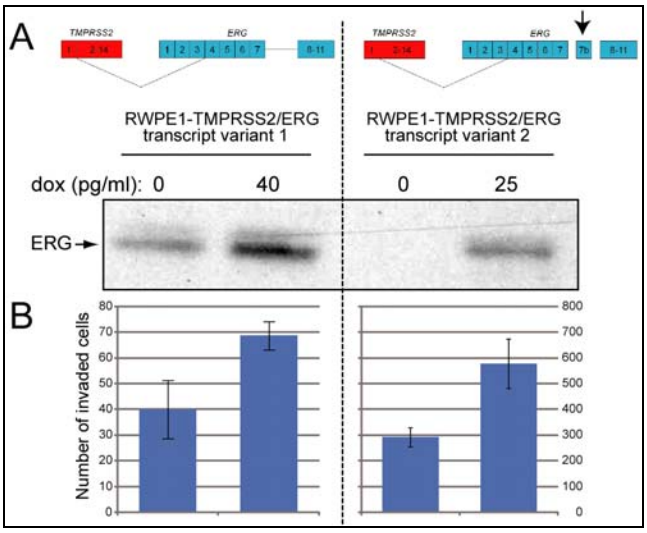

Fig 2. Conditional overexpression of a new TMPRSS2-ERG variant in RWPE1 cells. The original TMPRSS2/ERG cDNA (left) and a transcript variant (variant 2; right) containing an extra exon (7B, indicated in schematic above) were cloned into a tetracycline inducible vector, then stably transfected into a non-tumorigenic prostate epithelial line (RWPE1) harboring tet-repressor. In both lines, the TMPRSS2-ERG product is induced by doxycycline (Western blot; panel **A**), which promotes increased cell invasion (Boyden chamber invasion assay; panel **B**).

Task 1B:

We originally proposed to carry out a whole-genome pooled shRNA screen of 8 prostate cancer cell lines (4 with and 4 without ETS fusions). Towards this goal, we have carried out whole-genome shRNA (21,000 pooled shRNAs; purchased from Open Biosytems) biologic replicate screens of two different cell lines, RWPE1-TMPRSS2/ERG variant 1 and RWPE1-TMPRSS2/ERG variant 2. Following infection and selection of shRNA integrants, each replicate pool is split in two, and half the cells treated with doxycycline to induce TMPRSS2-ERG. After 4 weeks of growing out cells, shRNA barcodes are then PCR-amplified and hybridized to a barcode microarray, using the input shRNA library as the comparison reference. In addition, for the transcript variant 1 screen, we carried out technical replicates of the PCR-amplification and microarray hybridization steps, to help assess reproducibility. Results from these screens are summarized in Fig.3.

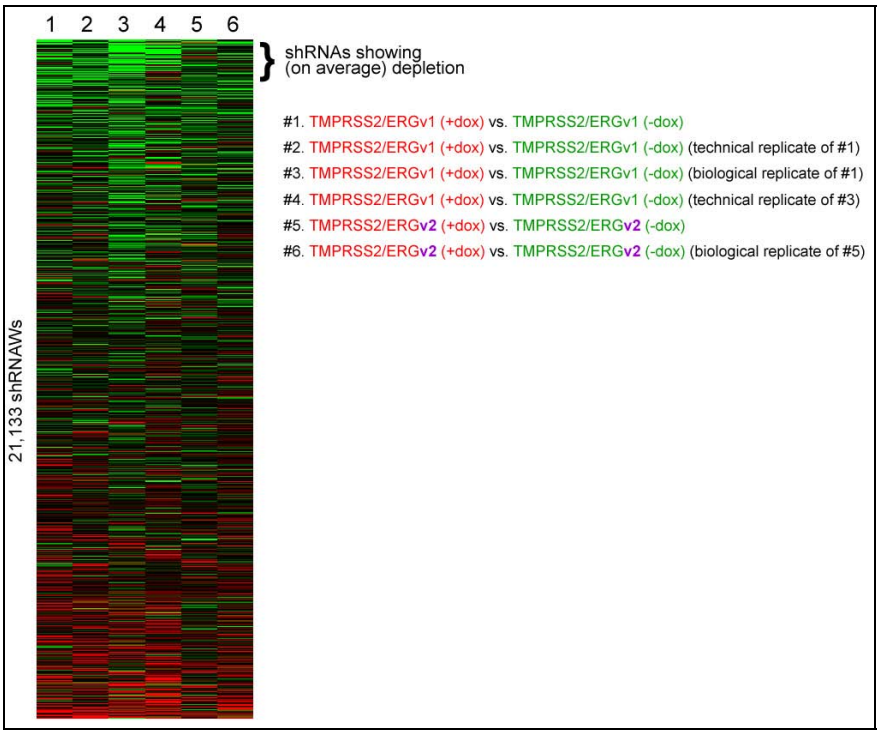

Fig 3. Whole-genome shRNA screens. Heatmap summarizes results of whole-genome (21,000) shRNA screens of RWPE1-TMPRSS2/ERG variant 1 (v1) and variant 2 (v2). Each of the six columns is a separate screen/microarray. Each row is a different shRNA, ordered by magnitude of average depletion (green) and enrichment (red). shRNAs exhibiting, on average, depletion are bracketed.

However, despite that shRNAs that on average exhibit depletion can be identified, the biologic replicates are not very reproducible (Fig. 4A). In our experience, we are able to obtain much more highly reproducible results using lower-complexity shRNA libraries, e.g. as shown in a screen of 570 shRNAs (Fig. 4B). This finding suggests that achieving and maintaining a high representation of shRNAs is problematic in the full genome screen.

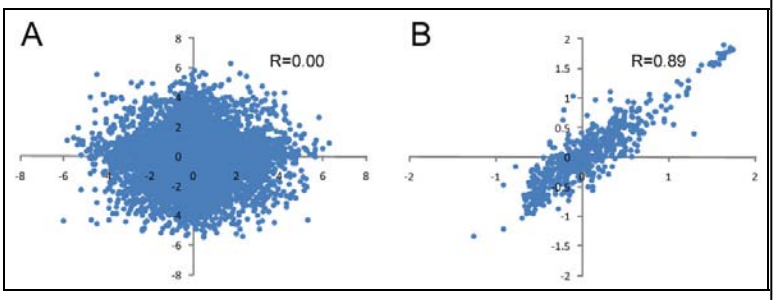

Fig 4. Reproducibility of shRNA screens. Shown are scatter plots of biological replicates (i.e. independent shRNA infections/selections) of **(A)** full - genome (21,000) shRNA screen of RWPE1-TMRSS2/ERG variant 1; and **(B)** focused shRNA screen, here using 570 shRNAs targeting pancreatic cancer genes in Panc5 cell line (funded separately). Higher reproducibility in the latter screen is evident by the X=Y scatter and the higher R value.

To circumvent this problem, we are moving to initiate a more targeted screen. Such a screen would include shRNAs targeting genes which we identify from microarray studies (both our own and from others) to be co-regulated with TMPRSS2-ERG in prostate tumor samples, as well as genes altered following TMPRSS2-ERG overexpression or depletion in cultured prostate cells. Priority would be placed on genes encoding enzymes and cell surface proteins, as these would be better drug targets. While the full-genome library is a non-renewable resource (purchased as virus from Open Biosystems), a focused screen would be renewable (purchased as shRNA plasmid clones). The focused screen would therefore permit us to carry out infections at much higher shRNA representation (i.e. number of infected cells carrying each shRNA), to perform many more replicates, and even to screen more cell lines, all at comparable costs.

Task 2: Analysis of the screen data to identify best candidate synthetic lethal targets will await completion of aim 1. We note that analysis should proceed quickly once the dataset is in hand.

Task 2: Individual target validation will await completion of aims 1 and 2.

Key Research Accomplishments

- Creation of LNCaP cell line with stable TMPRSS2-ERG knockdown, and creation of a second RWPE1 conditional cell line with inducible expression of a new TMPRSS2-ERG transcript variant.

- Completion of whole-genome shRNA screen in biologic duplicate of two prostate lines, and analysis of the resultant data.

Reportable Outcomes

- Studies during the first year of funding have not yet led to any manuscripts, abstract presentations, patents, or degrees granted.

- However, our piloting and optimizing of RNAi screens has been incorporated as preliminary data in grant applications, including a competitive renewal of R01 CA97139 ("Genomic instability in breast cancer").

Conclusion

In the first year of funding, we have made significant progress toward our goal to identify therapeutic targets displaying synthetic lethal (or Achilles' heel) interaction with ETS fusions in prostate cancer. Cell line reagents have been created, shRNA screening protocols optimized, and the first two whole-genome screens completed. Concerns of data reproducibility are leading us to consider initiating a more focused shRNA screen, either in parallel with or in place of full-genome screens.

References

None

Appendix

None